

8-Hydroxydeoxyguanosine in Human Leukocyte DNA and Daily Health Practice Factors: Effects of Individual Alcohol Sensitivity

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A typical oxidative DNA damage, 8-hydroxydeoxyguanosine (8-OHdG), was evaluated in human polymorphonuclear leukocytes (PMN) and mononuclear leukocytes (MN) by an anaerobic determination method. The mean 8-OHdG values were the lowest level ever reported [PMN, 3.07 ± 1.45 ; MN, 2.37 ± 1.21 8-OHdG/ 10^6 deoxyguanosine molecules (dG); $n = 92$]. According to a self-administered questionnaire to 92 healthy male workers, the relationship was investigated between 8-OHdG in leukocytes and daily health practice factors, that is, the frequency of physical exercise, smoking status, alcohol drinking, nutritional balance, and the degree of mental stress. A significant difference was observed only in alcohol drinking in subjects classified by aldehyde-dehydrogenase 2 isozyme (ALDH2) genotype. Habitual alcohol intake appeared to increase 8-OHdG in PMN from ALDH2-deficient subjects. Neither age, body mass index, nor any other factors examined showed any significant correlation with the 8-OHdG levels in leukocytes. **Key words:** alcohol drinking, ALDH2, 8-hydroxydeoxyguanosine, health practice factors, mononuclear leukocytes, polymorphonuclear leukocytes. *Environ Health Perspect* 104:1336–1338 (1996)

Among various kinds of DNA damage used as markers for cancer development, 8-hydroxydeoxyguanosine (8-OHdG), an oxidative adduct form of deoxyguanosine (dG), is considered to be one of the most sensitive (1). 8-OHdG is induced by several carcinogens and tumor promoters (2–6) and causes mutation both *in vitro* and *in vivo* (7–9). 8-OHdG has been evaluated in human leukocytes to elucidate correlations between 8-OHdG levels and etiological factors in patients with autoimmune diseases (10). Effects of health practice factors like physical exercise (11) and smoking (12,13) on 8-OHdG levels have also been investigated.

8-OHdG values vary considerably among those studies; thus, it is difficult to elucidate the correlation between those factors and the cellular 8-OHdG induction mechanism. We have established an anaerobic 8-OHdG determination method (14) that enables an accurate determination of 8-OHdG using human leukocytes, and we found this method suitable for human population studies using cellular 8-OHdG as a marker of cancer risk. By the method, we investigated the effects of daily health practice factors on 8-OHdG levels in leukocytes, i.e., both polymorphonuclear leukocytes (PMN) and mononuclear leukocytes (MN).

Materials and Methods

Subjects. Subjects were recruited from male workers in a factory during an annual health examination. After obtaining their consent, samples were collected from 92 male workers. We asked each worker to answer a self-administered questionnaire regarding their daily health practices.

Samples. Blood (7 ml) was collected into an EDTA-2Na-containing vessel. PMN and MN cells were separated with Mono-poly resolving medium (Dainippon Seiyaku, Osaka, Japan) within 4 hr after the sample collection. The remaining red blood cells in each cell fraction were removed by hypotonic lysis. Cells were washed with Dulbecco's phosphate buffered saline and stored as pellets at -80°C until assay.

8-OHdG assay. DNA extraction from cells and subsequent DNA digestion were carried out inside an anaerobic incubator as described previously (14). 8-OHdG was assayed using HPLC with electrochemical detection (15). The 8-OHdG value was calculated as 8-OHdG/ 10^6 dG, which was simultaneously assayed with a UV detector.

ALDH2 genotyping. To determine the individual sensitivity to an alcohol metabolite, acetaldehyde, the aldehyde-dehydrogenase 2 isozyme (ALDH2) genotypes of 82 subjects were examined using the nonisotopic polymerase chain reaction method described previously (16). ALDH2 is a mitochondrial enzyme that is highly efficient in detoxifying acetaldehyde to a less toxic metabolite, acetate. Human ALDH2 cDNA has been cloned and a single point mutation has been found in ALDH2-deficient people (17). The three ALDH2 genotypes have been characterized in the Japanese population: the typical homozygote ALDH2¹/ALDH2¹, the heterozygote ALDH2¹/ALDH2², and the atypical homozygote ALDH2²/ALDH2² (18). Acetaldehyde is quickly metabolized in people with ALDH2¹/ALDH2¹, but those with ALDH2¹/ALDH2² or ALDH2²/

ALDH2² have lower or no acetaldehyde metabolizing ability. ALDH2-deficient people, ALDH2¹/ALDH2² and ALDH2²/ALDH2², are known to be severely affected by acetaldehyde remaining in blood (18).

Statistical analysis. Statistical analysis was carried out using an SPSS statistical package (SPSS Inc., Chicago, IL) on the Osaka University ACOS-6 NEC computer system. The significance of the difference was analyzed by *t*-test (between two groups) and among more than two groups by one-way analysis of variance (ANOVA) test. The bivariate correlations of continuous variables were analyzed by the Pearson correlation coefficient.

Results

Table 1 shows the descriptive data on age, body mass index (BMI), and 8-OHdG in leukocytes of the 92 subjects. The difference of 8-OHdG between PMN and MN was statistically significant. There was a weak correlation between 8-OHdG in PMN and that in MN ($r = 0.205$; $p = 0.05$). Analysis by the Pearson's coefficient revealed that neither 8-OHdG in PMN nor that in MN showed correlation with age or with BMI (data not shown).

According to the self-administered questionnaire, subjects were classified on the basis of the five health practice factors: the frequency of physical exercise, smoking status, alcohol drinking (DRINK), awareness of nutritional balance, and the degree of subjective mental stress (Table 2). None of the factors showed any significant difference of 8-OHdG levels between groups in either PMN or MN.

No significant difference in 8-OHdG levels was observed among the three groups of alcohol drinking status (Table 3). Next, we compared the 8-OHdG level in the groups of subjects classified by ALDH2 genotype (Table 3). Here, to obtain a sufficient number of subjects for analysis, the difference was analyzed between DRINK-everyday groups and DRINK-not-everyday (including "less than five times per week")

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and “none” subjects) groups. None of the ALDH2²/ALDH2² were habitual drinkers. Comparing 8-OHdG between DRINK-everyday and DRINK-not-everyday groups in each genotype, the DRINK-everyday groups showed higher levels than the DRINK-not-everyday groups both in PMN and in MN. In PMN from ALDH2¹/ALDH2² subjects, 8-OHdG of the DRINK-everyday group was significantly higher than that of the DRINK-not-everyday group. There was a significant correlation between 8-OHdG in PMN and the daily amount of alcohol intake in ALDH2¹/ALDH2² subjects (Fig. 1). In MN, no correlation was observed in ALDH2¹/ALDH2¹ ($r = -0.018$) or ALDH2¹/ALDH2² subjects ($r = 0.085$).

Discussion

We evaluated 8-OHdG in human leukocytes by the anaerobic 8-OHdG determination method, which has made the determination more accurate and reproducible than before (14). The mean 8-OHdG was the lowest level ever reported. The difference between 8-OHdG in PMN and MN was statistically significant (Table 1), and there was a weak correlation between 8-OHdG values in PMN and MN when analyzed by the Pearson's coefficient. The present findings are in agreement with the study by Bashir et al. (10), although 8-OHdG in our study showed 1/60–1/30 lower levels than the previous study. However, the 8-OHdGs of the two cell types did not always show the same difference with each subject. One third of the subjects ($n = 29$) showed higher 8-OHdG in MN than PMN. We could not find any factor in this study that might correlate the ratio of 8-OHdG in PMN to that in MN. There might be an accidental oxidative stress generation during leukocyte separation and the following procedure in the cell preparation, even though we had carefully controlled the temperature to minimize the effect of the accidental factors. In PMN, which are known to easily generate reactive oxygen species against various kinds of stimuli, the accidental 8-OHdG formation might strongly occur. Further inquiries are needed to verify the difference of 8-OHdG between PMN and MN, especially in factors associated with oxidative stress.

Among the factors investigated, the effect of alcohol drinking on 8-OHdG was observed when subjects were classified by ALDH2 genotype (Table 3). In ALDH2¹/ALDH2² subjects, 8-OHdG in PMN of the DRINK-everyday group was significantly higher than that in the DRINK-not-everyday group. Moreover, the amount of alcohol intake showed a correlation with 8-OHdG in PMN from ALDH2¹/ALDH2²

Table 1. Descriptive data of 92 subjects

	Mean \pm SD	Minimum	Maximum
Age	42.0 \pm 9.1	20	59
BMI	22.5 \pm 2.8	17.3	34.0
8-OHdG/10 ⁶ dG			
PMN	3.07 \pm 1.45 ^a	0.63	7.52
MN	2.37 \pm 1.21 ^a	0.74	7.84

Abbreviations: BMI, body mass index; 8-OHdG/10⁶dG, 8-hydroxydeoxyguanosine per 10⁶ deoxyguanosine; PMN, polymorphonuclear leukocytes; MN, mononuclear leukocytes.

^aPMN is significantly higher than MN ($p < 0.001$ by paired t -test).

Table 2. Grouping of the subjects by health practice factors

Health practice factor	Group
Physical exercise	Almost everyday Less than four times/week None
Cigarette smoking	Smoker Ex-smoker Nonsmoker
Alcohol drinking	Almost everyday Less than five times/week None
Nutritional balance	Aware of Sometimes or not aware of
Subjective mental stress	Much Normal or little

Table 3. The effects of alcohol drinking and ALDH2 genotype on 8-OHdG

		8-OHdG/10 ⁶ dG (mean \pm SD)		
		No.	PMN	MN
With whole subjects	Everyday	31	3.37 \pm 1.60	2.45 \pm 0.91
	Less than five times/week	48	2.86 \pm 1.28	2.32 \pm 1.43
	None	13	3.14 \pm 1.62	2.40 \pm 1.01
ALDH2 genotype				
	ALDH2 ¹ /ALDH2 ¹			
	Everyday	20	2.93 \pm 1.42	2.37 \pm 0.95
	Not everyday	16	2.39 \pm 1.36	2.39 \pm 1.31
ALDH2 ¹ /ALDH2 ²	Everyday	9	4.19 \pm 1.84 ^a	2.56 \pm 0.95
	Not everyday	31	2.89 \pm 1.37 ^a	2.19 \pm 1.47
ALDH2 ² /ALDH2 ²	Not everyday	6	3.48 \pm 1.22	3.21 \pm 0.95

Abbreviations: PMN, polymorphonuclear leukocytes; MN, mononuclear leukocytes.

^aThe everyday group is significantly higher than the not-everyday group ($p < 0.05$).

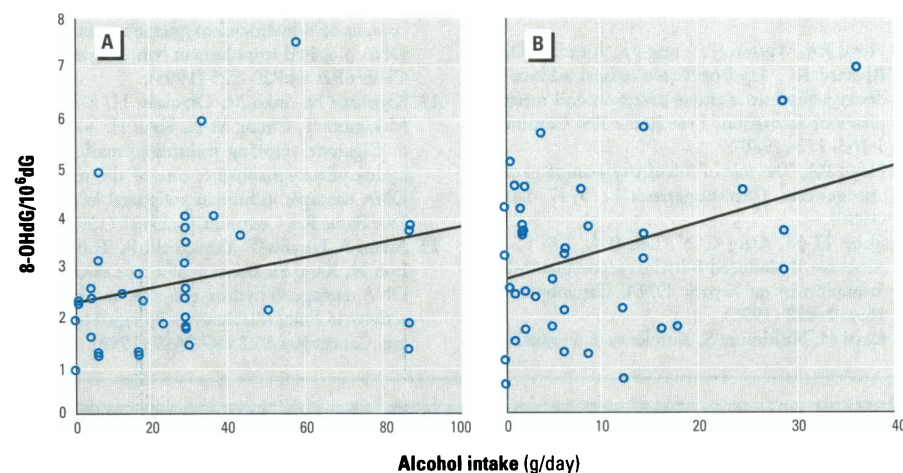


Figure 1. The correlation between the daily amount of alcohol intake and 8-OHdG in polymorphonuclear leukocytes with (A) ALDH2¹/ALDH2¹ subjects ($n = 36$) and with (B) ALDH2¹/ALDH2² subjects ($n = 40$). ALDH2¹/ALDH2¹: $r = 0.276$, $p = 0.103$; ALDH2¹/ALDH2²: $r = 0.351$, $p = 0.026$.

subjects, and the effect was stronger in the ALDH2¹/ALDH2² subjects than in the ALDH2¹/ALDH2¹ subjects (Fig. 1).

ALDH2 is an enzyme responsible for the individual sensitivity to alcohol (16,18) as described in Materials and Methods. Because ALDH2-deficient genotypes (ALDH2¹/ALDH2² and ALDH2²/ALDH2²) have higher acetaldehyde accumulation in blood, they may be susceptible to the harmful influence of alcohol via toxic

effects of acetaldehyde. 8-OHdG formation is considered to be correlated with free radical generation, and contribution of acetaldehyde-induced free radicals to alcohol-related health problems has been suggested (19). Our findings may show that individual sensitivity, as well as the frequency of alcohol exposure, is an important factor in the mechanism of alcohol-related health problems such as cancer development.

The health practice factors investigated have been reported to have correlation with 8-OHdG in human lymphocytes (11), leukocytes (12,13), and urine (20,21) and in an *in vivo* study using rats (22). Age (23,24) and BMI (21) have also been reported to be correlated with 8-OHdG levels in human tissues and in urine, respectively. On the contrary, with those factors, we failed to detect any significant difference of 8-OHdG in leukocytes. The oxidative damage appearing in leukocytes from healthy donors might be milder than that in tissues, which may be the target of cancer development due to oxidative stress. Moreover, PMN, the role of which is the first-line-defense against foreign organisms, might be more strongly affected by factors that can cause transient respiratory burst than the accumulative effect of daily oxidative stress.

In conclusion, we evaluated 8-OHdG in PMN and MN, with the lowest value ever reported. However, 8-OHdG in leukocytes from healthy male subjects did not show any significant correlation with the health practice factors investigated, except for the effect of alcohol drinking on the subjects who were genetically susceptible to the effect of acetaldehyde.

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Postdoctoral Position Molecular Epidemiology

A postdoctoral position at the Harvard School of Public Health is available to study susceptibility to the toxic effects of lead. Laboratory experience in genotyping and a background in the biology of lead and heavy metal toxicity, as well as a working knowledge of epidemiology and biostatistics, is preferred. Send a C.V. and names of three references to Dr. Karl T. Kelsey, Department of Environmental Health, Harvard School of Public Health, 65 Huntington Ave, Boston, MA 02115.